

In the Claims

1. (Currently amended) An isolated nucleic acid molecule ~~that regulates the expression of a cold shock inducible gene under physiological conditions which cause the cold shock response in a bacterium, wherein said isolated nucleic acid molecule consists~~ consisting essentially of nucleotides 1-11 of SEQ. ID NO:55, nucleotides 56-117 of SEQ. ID NO:55, nucleotides 123-135 of SEQ. ID NO:55, SEQ. ID NO:49, or SEQ. ID NO:50.

Claims 2-4 (Canceled)

5. (Previously presented) The nucleic acid molecule of Claim 1 consisting essentially of nucleotides +1 to +11 of the *cspA* 5'-UTR (nucleotides 1 to 11 of SEQ. ID. NO. 55).

6. (Currently amended) The isolated nucleic acid molecule of Claim 1, ~~wherein said cold shock inducible gene~~ which interacts with CspA protein.

7-9. (Cancelled)

10. (Previously presented) The nucleic acid molecule of Claim 1 consisting essentially of nucleotides +56 to +117 of the *cspA* 5'-UTR (nucleotides 56 to 117 of SEQ. ID. No. 55).

11-13. (Cancelled)

14. (Previously presented) The nucleic acid molecule of Claim 1 consisting essentially of nucleotides +123 to +135 of the *cspA* 5'-UTR (nucleotides 123 to 135 of SEQ. ID. NO. 55).

15. (Previously presented) The nucleic acid molecule of Claim 1 consisting essentially of a sequence selected from the group consisting of SEQ ID NO:49 and SEQ ID NO:50.

16. (Currently amended) A nucleic acid vector that enhances translation of a gene transcript under conditions that elicit a cold-shock response in a bacterium, comprising a downstream box, a sequence coding for said transcript, and a first nucleic acid fragment comprising SEQ. ID NO:48, SEQ. ID NO:49, SEQ. ID NO:50 or a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to SEQ. ID NO:48, SEQ. ID NO:49 or SEQ. ID NO:50, wherein said first nucleic acid fragment is derived from a first nucleic acid molecule comprising ~~said first nucleic~~

~~acid fragment~~ and a first cold shock inducible gene having a protein coding region, wherein said first nucleic acid fragment enhances translation of said ~~first cold shock inducible gene~~ transcript under conditions that elicit the cold shock response in bacterium, and wherein said nucleic acid vector ~~is free from said~~ does not contain the entire protein coding region of the first cold shock inducible gene.

17. (Previously presented) The nucleic acid vector of Claim 16 further comprising a Shine-Dalgarno sequence.

18. (Currently amended) A nucleic acid vector of Claim 16 further comprising a cold box, wherein said vector ~~directs prolonged expression and~~ enhances translation of a gene transcript and directs prolonged production of a protein encoded by the transcript under conditions that elicit a cold shock response in a bacterium.

19. (Currently amended) A nucleic acid vector that ~~directs the prolonged expression and~~ enhances the translation of a gene transcript under conditions of physiological stress that elicit a cold shock response of a bacterium, and represses the expression of the gene product under physiological conditions comprising:

a first nucleic acid fragment comprising SEQ. ID NO:48, SEQ. ID NO:49, SEQ. ID NO:50 or a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to any of them, said first nucleic acid fragment being derived from a first nucleic acid molecule comprising said ~~first nucleic acid fragment~~ and a first cold shock inducible gene having a protein coding region, wherein said first nucleic acid fragment enhances translation of said first cold shock inducible gene under cold shock conditions;

a second nucleic acid fragment comprising nucleotides 56-117 of SEQ. ID NO:55 or a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to nucleotides 56-117 of SEQ. ID NO:55; derived from said first nucleic acid molecule or from a second nucleic acid molecule, said second nucleic acid molecule comprising said second nucleic acid fragment and a second cold shock inducible gene, wherein said second nucleic acid fragment represses expression of said first or second cold shock inducible gene under physiological conditions;

a cold box comprising nucleotides 1-11 of SEQ. ID NO:55 or a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to nucleotides 1-11 of SEQ. ID NO:55[[,]]; and

a downstream box[[,]];

wherein said vector ~~is free from said~~ does not contain the entire protein coding region of the first cold shock inducible gene.

20. (Currently amended) The vector of claim 16, wherein the sequence coding for said transcript is derived from ~~further comprising a coding region of~~ a second cold shock inducible gene.

21. (Currently amended) The vector of Claim 18, wherein the sequence coding for said transcript is derived from ~~further comprising a coding region of~~ a second cold-shock inducible gene.

22. (Currently amended) The vector of Claim 19, wherein said second nucleic acid fragment is derived from a second nucleic acid molecule, and wherein the vector further comprising comprises a coding region of a third cold-shock inducible gene, ~~wherein when said first nucleic acid fragment is derived from said first nucleic acid molecule and said second nucleic acid fragment is derived from said second nucleic acid molecule, said third cold-shock inducible gene may be one of said first cold shock inducible gene or said second cold shock inducible gene.~~

23. (Previously presented) The vector of Claim 16 further comprising a coding region of a heterologous gene.

24. (Previously presented) The vector of Claim 18 further comprising a coding region of a heterologous gene.

25. (Previously presented) The vector of Claim 19, further comprising a coding region of a heterologous gene.

26. (Previously presented) The vector of Claim 16, further comprising a promoter and at least one restriction site downstream of said first nucleic acid fragment and said downstream box for inserting an additional DNA fragment.

27. (Previously presented) The vector of Claim 18, further comprising a promoter and at least one restriction site downstream of said cold box, said first nucleic acid fragment, and said downstream box for inserting an additional DNA fragment.

28. (Currently amended) A nucleic acid vector that ~~directs prolonged expression and~~ enhances translation of a gene transcript under conditions of physiological stress that elicit a cold shock response of a bacterium, and represses expression of the gene product under physiological conditions comprising:

a first nucleic acid fragment comprising SEQ. ID NO:48, SEQ. ID NO:49, SEQ. ID NO:50 or a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to SEQ. ID NO:48, SEQ. ID NO:49 or SEQ. ID NO:50, said first nucleic acid fragment being derived from a first nucleic acid molecule comprising ~~said first nucleic acid fragment and~~ a first cold shock inducible gene having a protein coding region; ~~wherein said first nucleic acid fragment enhances translation of said first cold shock inducible gene under cold shock conditions,~~

a second nucleic acid fragment comprising nucleotides 56-117 of SEQ. ID NO:55 or a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to nucleotides 56-117 of SEQ. ID NO:55; ~~derived from said first nucleic acid molecule or from a second nucleic acid molecule, said second nucleic acid molecule comprising said second nucleic acid fragment and a second cold shock inducible gene, wherein said second nucleic acid fragment represses expression of said first or second cold shock inducible gene under physiological conditions,~~

a cold box comprising nucleotides 1-11 of SEQ. ID NO:55 or a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to nucleotides 1-11 of SEQ. ID NO:55[[,]];

a downstream box[[,]];

a promoter; and

at least one restriction site downstream of said cold box, said first nucleic acid fragment, said second nucleic acid fragment, and said downstream box for inserting an additional DNA fragment.

29. (Previously presented) The vector of claim 26, wherein said additional DNA fragment comprises a coding region of a second cold shock inducible gene.

30. (Previously presented) The vector of Claim 27, wherein said additional DNA fragment comprises a coding region of a second cold shock inducible gene.

31. (Currently amended) The vector of Claim 28, wherein said additional DNA fragment comprises a coding region of a second cold shock inducible gene.

32. (Previously presented) The vector of Claim 26, wherein said additional DNA fragment comprises a coding region of a heterologous gene.

33. (Previously presented) The vector of Claim 27, wherein said additional DNA fragment comprises a coding region of a heterologous gene.

34. (Previously presented) The vector of Claim 28, wherein said additional DNA fragment comprises a coding region of a heterologous gene.

35. (Previously presented) A transformed bacteria containing the vector of Claim 16.

36. (Previously presented) A transformed bacteria containing the vector of Claim 18.

37. (Previously presented) A transformed bacteria containing the vector of Claim 19.

38. (Currently amended) A method for overexpressing a gene comprising the steps of:

transforming bacteria with a nucleic acid vector that enhances translation of a gene transcript under conditions that elicit a cold shock response in a bacterium comprising

a downstream box,

a first nucleic acid fragment comprising SEQ. ID NO:48, SEQ. ID NO:49, SEQ. ID NO:50 or a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to SEQ. ID NO:48, SEQ. ID NO:49 or SEQ. ID NO:50, said first nucleic acid fragment being derived from a first nucleic acid molecule comprising ~~said first nucleic acid fragment~~ and a first cold shock inducible gene, and

a gene protein coding region derived from a second nucleic acid molecule,

wherein said first nucleic acid fragment enhances translation of a transcript derived from said protein coding region ~~said first cold shock inducible gene~~ under cold shock conditions[.,,]; and

subjecting said bacteria to conditions that elicit a cold shock response.

39. (Currently amended) A method for overexpressing a gene comprising the steps of:

transforming bacteria with a nucleic acid vector comprising:

a downstream box,

a cold box comprising nucleotides 1-11 of SEQ. ID NO:55 or a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to nucleotides 1-11 of SEQ. ID NO:55,

a first nucleic acid fragment comprising SEQ. ID NO:48, SEQ. ID NO:49, SEQ. ID NO:50 or a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to SEQ. ID NO:48, SEQ. ID NO:49 or SEQ. ID NO:50, said first nucleic acid fragment being derived from a first nucleic acid molecule comprising ~~said first nucleic acid fragment~~ and a first cold shock inducible gene having a first protein coding region, and

a ~~gene~~ a second protein coding region derived from a second nucleic acid molecule,

wherein said vector ~~directs prolonged expression and~~ enhances translation of a transcript derived from said protein coding region ~~a gene~~ under conditions that elicit a cold shock response in a bacterium[[,]]; and

subjecting said bacteria to conditions that elicit a cold shock response.

40. (Currently amended) A method for overexpressing a gene comprising the steps of:

transforming bacteria with a nucleic acid vector comprising

a first nucleic acid fragment comprising SEQ. ID NO:48, SEQ. ID NO:49, SEQ. ID NO:50 or a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to SEQ. ID NO:48, SEQ. ID NO:49 or SEQ. ID NO:50, said first nucleic acid fragment being derived from a first nucleic acid molecule comprising ~~said first nucleic acid fragment~~ and a first cold shock inducible gene; ~~wherein said first nucleic acid fragment enhances translation of said first cold shock inducible gene,~~

a second nucleic acid fragment comprising nucleotides 56-117 of SEQ. ID NO:55 or a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to nucleotides 56-117 of SEQ. ID NO:55 ~~derived from said first nucleic acid molecule or from a second nucleic acid molecule, said second nucleic acid molecule comprising said second nucleic acid fragment and a second cold shock inducible gene, wherein said second nucleic acid fragment represses expression of the second cold shock inducible gene,~~

a cold box comprising nucleotides 1-11 of SEQ. ID NO:55 or a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to nucleotides 1-11 of SEQ. ID NO:55,

a downstream box, and

a protein coding region derived from a second nucleic acid molecule,

wherein said vector ~~directs prolonged expression and~~ enhances translation of a transcript derived from the protein coding region ~~a gene~~ under conditions that elicit a cold shock response of a bacterium and represses expression of the protein coding region ~~gene~~ under physiological conditions[[,]]; and

subjecting said bacteria to conditions that elicit a cold shock response.

41. (Previously presented) The method of Claim 40, wherein said overexpression causes a reduction in the expression of at least one endogenous protein.

42. (Previously presented) The method of Claim 38, wherein said overexpression causes a reduction in the expression of at least one endogenous protein.

43. (Previously presented) The method of claim 39, wherein said overexpression causes a reduction in the expression of at least one endogenous protein.

44. (Previously presented) The method of Claim 40, wherein said conditions that elicit a cold shock response comprise subjecting said bacteria to a sufficiently low temperature to elicit a cold-shock response.

45. (Previously presented) The method of Claim 38, wherein said conditions that elicit a cold shock response comprise subjecting said bacteria to a sufficiently low temperature to elicit a cold-shock response.

46. (Previously presented) The method of Claim 39, wherein said conditions that elicit a cold shock response comprise subjecting said bacteria to a sufficiently low temperature to elicit a cold-shock response.

47. (Previously presented) The method of Claim 46, wherein said temperature is about 10-15°C.

48. (Previously presented) The method of Claim 44, wherein said temperature is about 10-15°C.

49. (Previously presented) The method of Claim 45, wherein said temperature is about 10-15°C.

50. (Currently amended) A vector capable of expressing a heterologous gene in a bacterium at physiological temperature or under conditions that elicit a cold shock response comprising ~~regulatory elements~~ in the following order: a promoter, at least a portion of a 5'-UTR of a cold shock inducible gene, a Shine-Dalgarno sequence, a translation initiation codon, a downstream box, and at least one restriction enzyme recognition site for insertion of ~~said a heterologous gene protein coding region, wherein said portion of the 5'-UTR of a cold shock inducible gene is a regulatory element selected from the group consisting of nucleotides 1-11 of SEQ. ID NO:55, nucleotides 56-117 of SEQ. ID NO:55, nucleotides 123-135 of SEQ. ID NO:55, SEQ. ID NO:49, SEQ. ID NO:50 and a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to nucleotides 1-11 of SEQ. ID NO:55, nucleotides 56-117 of SEQ. ID NO:55, nucleotides 123-135 of SEQ. ID NO:55, SEQ. ID NO:49 or SEQ. ID NO:50.~~

51. (Currently amended) The vector of Claim 50, further comprising ~~an additional nucleic acid fragment~~ a coding region for a heterologous protein inserted at said restriction enzyme recognition site, wherein said ~~fragment~~ coding region is derived from ~~comprises a coding region of a cold shock inducible gene and wherein said fragment is regulated by one or more of said regulatory elements.~~

52. (Currently amended) The vector of Claim 50, further comprising ~~an additional nucleic acid fragment~~ a coding region for a heterologous protein inserted at said restriction enzyme site, wherein said ~~fragment~~ coding region comprises a coding region of a heterologous gene and ~~wherein said fragment is regulated by said regulatory elements.~~

53. (Previously presented) A transformed bacteria containing the vector of Claim 50.
54. (Previously presented) A method of overexpressing a gene comprising transforming bacteria with a nucleic acid vector of Claim 51 or Claim 52 and subjecting said bacteria to conditions that elicit a cold shock response.
55. (Previously presented) The method of Claim 54, wherein said conditions that elicit a cold shock response comprise subjecting said bacteria to a sufficiently low temperature to elicit a cold-shock response.
56. (Previously presented) The method of claim 55, wherein said temperature is about 10-15°C.
57. (Currently amended) An isolated nucleic acid molecule competent to prolong the expression of a cold shock gene during adaptation of a bacterium to physiological stress which elicits a cold shock response, said nucleic acid molecule consisting essentially of between 8 and 25 sequential nucleotides of nucleotides 1-25 of SEQ. ID NO:55, or a fragment that will hybridize under low or high stringency conditions to a reference nucleic acid molecule that is precisely complementary to between 8 and 25 sequential nucleotides of nucleotides 1-25 of SEQ. ID NO:55 ~~the first 25 nucleotides of a 5' UTR of a cold shock inducible mRNA transcript.~~
58. (New) The nucleic acid vector of claim 16 wherein the
low stringency conditions for hybridization comprise
pretreating filters containing the reference nucleic acid molecule for 6 hours at 40°C in a pre-hybridization solution of 35% formamide, 5xSSC, 50 mM Tris-HCL (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, 500 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate,
adding 5-20 x 10⁶ cpm ³²P-labeled probe to the pre-hybridization solution to form a hybridization mixture,
incubating the filters in the hybridization mixture for 18-20 hours at 40°C,
washing the filters for 1.5 hours at 55°C in a wash solution containing 2xSSC, 25 mM Tris-HCL (pH 7.4), 5 mM EDTA, and 0.1% SDS, and
replacing the wash solution with fresh wash solution of the same composition and incubating the filters for an additional 1.5 hours at 60°C; and

wherein the high stringency conditions for hybridization use a method comprising the steps of

providing a subject nucleic acid molecule for which the ability to hybridize to the precisely complimentary fragment is to be determined,

pretreating filters containing the reference nucleic acid molecule for 8 hours or more at 65°C in buffer containing 6xSSC, 50 mM Tris-HCL (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA and 500 µg/ml denatured salmon sperm DNA,

washing the filters,

incubating the filters for 48 hours at 65°C in a hybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 x 10⁶ ³²P-labeled probe,

washing the filters for 1 hour in a solution of 2xSSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA at 37°C, and

washing the filters for 45 minutes in 0.1xSSC at 50°C.

59. (New) The nucleic acid vector of claim 19 wherein the low stringency conditions for hybridization comprise

pretreating filters containing the reference nucleic acid molecule for 6 hours at 40°C in a pre-hybridization solution of 35% formamide, 5xSSC, 50 mM Tris-HCL (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, 500 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate,

adding 5-20 x 10⁶ cpm ³²P-labeled probe to the pre-hybridization solution to form a hybridization mixture,

incubating the filters in the hybridization mixture for 18-20 hours at 40°C,

washing the filters for 1.5 hours at 55°C in a wash solution containing 2xSSC, 25 mM Tris-HCL (pH 7.4), 5 mM EDTA, and 0.1% SDS, and

replacing the wash solution with fresh wash solution of the same composition and incubating the filters for an additional 1.5 hours at 60°C; and

wherein the high stringency conditions for hybridization use a method comprising the steps of

providing a subject nucleic acid molecule for which the ability to hybridize to the precisely complimentary fragment is to be determined,

pretreating filters containing the reference nucleic acid molecule for 8 hours or more at 65°C in buffer containing 6xSSC, 50 mM Tris-HCL (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA and 500 µg/ml denatured salmon sperm DNA,

washing the filters,

incubating the filters for 48 hours at 65°C in a hybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 x 10⁶ ³²P-labeled probe,

washing the filters for 1 hour in a solution of 2xSSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA at 37°C, and

washing the filters for 45 minutes in 0.1xSSC at 50°C.

60. (New) The nucleic acid vector of claim 28 wherein the low stringency conditions for hybridization comprise

pretreating filters containing the reference nucleic acid molecule for 6 hours at 40°C in a pre-hybridization solution of 35% formamide, 5xSSC, 50 mM Tris-HCL (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, 500 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate,

adding 5-20 x 10⁶ cpm ³²P-labeled probe to the pre-hybridization solution to form a hybridization mixture,

incubating the filters in the hybridization mixture for 18-20 hours at 40°C,

washing the filters for 1.5 hours at 55°C in a wash solution containing 2xSSC, 25 mM Tris-HCL (pH 7.4), 5 mM EDTA, and 0.1% SDS, and

replacing the wash solution with fresh wash solution of the same composition and incubating the filters for an additional 1.5 hours at 60°C; and

wherein the high stringency conditions for hybridization use a method comprising the steps of

providing a subject nucleic acid molecule for which the ability to hybridize to the precisely complimentary fragment is to be determined,

pretreating filters containing the reference nucleic acid molecule for 8 hours or more at 65°C in buffer containing 6xSSC, 50 mM Tris-HCL (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA and 500 µg/ml denatured salmon sperm DNA,

washing the filters,

incubating the filters for 48 hours at 65°C in a hybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 x 10⁶ ³²P-labeled probe,

washing the filters for 1 hour in a solution of 2xSSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA at 37°C, and

washing the filters for 45 minutes in 0.1xSSC at 50°C.

61. (New) The nucleic acid vector of claim 38 wherein the low stringency conditions for hybridization comprise

pretreating filters containing the reference nucleic acid molecule for 6 hours at 40°C in a pre-hybridization solution of 35% formamide, 5xSSC, 50 mM Tris-HCL (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, 500 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate,

adding 5-20 x 10⁶ cpm ³²P-labeled probe to the pre-hybridization solution to form a hybridization mixture,

incubating the filters in the hybridization mixture for 18-20 hours at 40°C,

washing the filters for 1.5 hours at 55°C in a wash solution containing 2xSSC, 25 mM Tris-HCL (pH 7.4), 5 mM EDTA, and 0.1% SDS, and

replacing the wash solution with fresh wash solution of the same composition and incubating the filters for an additional 1.5 hours at 60°C; and

wherein the high stringency conditions for hybridization use a method comprising the steps of

providing a subject nucleic acid molecule for which the ability to hybridize to the precisely complimentary fragment is to be determined,

pretreating filters containing the reference nucleic acid molecule for 8 hours or more at 65°C in buffer containing 6xSSC, 50 mM Tris-HCL (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA and 500 µg/ml denatured salmon sperm DNA,

washing the filters,

incubating the filters for 48 hours at 65°C in a hybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 x 10⁶ ³²P-labeled probe,

washing the filters for 1 hour in a solution of 2xSSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA at 37°C, and

washing the filters for 45 minutes in 0.1xSSC at 50°C.

62. (New) The nucleic acid vector of claim 39 wherein the low stringency conditions for hybridization comprise

pretreating filters containing the reference nucleic acid molecule for 6 hours at 40°C in a pre-hybridization solution of 35% formamide, 5xSSC, 50 mM Tris-HCL (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, 500 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate,

adding 5-20 x 10⁶ cpm ³²P-labeled probe to the pre-hybridization solution to form a hybridization mixture,

incubating the filters in the hybridization mixture for 18-20 hours at 40°C,

washing the filters for 1.5 hours at 55°C in a wash solution containing 2xSSC, 25 mM Tris-HCL (pH 7.4), 5 mM EDTA, and 0.1% SDS, and

replacing the wash solution with fresh wash solution of the same composition and incubating the filters for an additional 1.5 hours at 60°C; and

wherein the high stringency conditions for hybridization use a method comprising the steps of

providing a subject nucleic acid molecule for which the ability to hybridize to the precisely complimentary fragment is to be determined,

pretreating filters containing the reference nucleic acid molecule for 8 hours or more at 65°C in buffer containing 6xSSC, 50 mM Tris-HCL (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA and 500 µg/ml denatured salmon sperm DNA,

washing the filters,

incubating the filters for 48 hours at 65°C in a hybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 x 10⁶ ³²P-labeled probe,

washing the filters for 1 hour in a solution of 2xSSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA at 37°C, and

washing the filters for 45 minutes in 0.1xSSC at 50°C.

63. (New) The nucleic acid vector of claim 40 wherein the low stringency conditions for hybridization comprise

pretreating filters containing the reference nucleic acid molecule for 6 hours at 40°C in a pre-hybridization solution of 35% formamide, 5xSSC, 50 mM Tris-HCL (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, 500 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate,

adding $5-20 \times 10^6$ cpm ^{32}P -labeled probe to the pre-hybridization solution to form a hybridization mixture,

incubating the filters in the hybridization mixture for 18-20 hours at 40°C,

washing the filters for 1.5 hours at 55°C in a wash solution containing 2xSSC, 25 mM Tris-HCL (pH 7.4), 5 mM EDTA, and 0.1% SDS, and

replacing the wash solution with fresh wash solution of the same composition and incubating the filters for an additional 1.5 hours at 60°C; and

wherein the high stringency conditions for hybridization use a method comprising the steps of

providing a subject nucleic acid molecule for which the ability to hybridize to the precisely complimentary fragment is to be determined,

pretreating filters containing the reference nucleic acid molecule for 8 hours or more at 65°C in buffer containing 6xSSC, 50 mM Tris-HCL (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA and 500 µg/ml denatured salmon sperm DNA,

washing the filters,

incubating the filters for 48 hours at 65°C in a hybridization mixture containing 100 µg/ml denatured salmon sperm DNA and $5-20 \times 10^6$ ^{32}P -labeled probe,

washing the filters for 1 hour in a solution of 2xSSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA at 37°C, and

washing the filters for 45 minutes in 0.1xSSC at 50°C.

64. (New) The nucleic acid vector of claim 50 wherein the low stringency conditions for hybridization comprise

pretreating filters containing the reference nucleic acid molecule for 6 hours at 40°C in a pre-hybridization solution of 35% formamide, 5xSSC, 50 mM Tris-HCL (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, 500 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate,

adding $5-20 \times 10^6$ cpm ^{32}P -labeled probe to the pre-hybridization solution to form a hybridization mixture,

incubating the filters in the hybridization mixture for 18-20 hours at 40°C ,

washing the filters for 1.5 hours at 55°C in a wash solution containing 2xSSC, 25 mM Tris-HCL (pH 7.4), 5 mM EDTA, and 0.1% SDS, and

replacing the wash solution with fresh wash solution of the same composition and incubating the filters for an additional 1.5 hours at 60°C ; and

wherein the high stringency conditions for hybridization use a method comprising the steps of

providing a subject nucleic acid molecule for which the ability to hybridize to the precisely complimentary fragment is to be determined,

pretreating filters containing the reference nucleic acid molecule for 8 hours or more at 65°C in buffer containing 6xSSC, 50 mM Tris-HCL (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA and 500 $\mu\text{g/ml}$ denatured salmon sperm DNA,

washing the filters,

incubating the filters for 48 hours at 65°C in a hybridization mixture containing 100 $\mu\text{g/ml}$ denatured salmon sperm DNA and $5-20 \times 10^6$ ^{32}P -labeled probe,

washing the filters for 1 hour in a solution of 2xSSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA at 37°C , and

washing the filters for 45 minutes in 0.1xSSC at 50°C .

65. (New) The nucleic acid vector of claim 57 wherein the low stringency conditions for hybridization comprise

pretreating filters containing the reference nucleic acid molecule for 6 hours at 40°C in a pre-hybridization solution of 35% formamide, 5xSSC, 50 mM Tris-HCL (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, 500 $\mu\text{g/ml}$ salmon sperm DNA, 10% (wt/vol) dextran sulfate,

adding $5-20 \times 10^6$ cpm ^{32}P -labeled probe to the pre-hybridization solution to form a hybridization mixture,

incubating the filters in the hybridization mixture for 18-20 hours at 40°C ,

washing the filters for 1.5 hours at 55°C in a wash solution containing 2xSSC, 25 mM Tris-HCL (pH 7.4), 5 mM EDTA, and 0.1% SDS, and

replacing the wash solution with fresh wash solution of the same composition and incubating the filters for an additional 1.5 hours at 60°C; and

wherein the high stringency conditions for hybridization use a method comprising the steps of

providing a subject nucleic acid molecule for which the ability to hybridize to the precisely complimentary fragment is to be determined,

pretreating filters containing the reference nucleic acid molecule for 8 hours or more at 65°C in buffer containing 6xSSC, 50 mM Tris-HCL (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA and 500 µg/ml denatured salmon sperm DNA,

washing the filters,

incubating the filters for 48 hours at 65°C in a hybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 x 10⁶ ³²P-labeled probe,

washing the filters for 1 hour in a solution of 2xSSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA at 37°C, and

washing the filters for 45 minutes in 0.1xSSC at 50°C.